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Mental Retardation in a Boy With an Interstitial Deletion at Xp22.3 Involving STS, KAL1, and OA1: Implication for the MRX Locus

Koji Muroya, Tsutomu Ogata, Nobutake Matsuo, Toshiro Nagai, Brunella Franco, Andrea Ballabio, Gudrun Rappold, Nobuo Sakura, and Yoshimitsu Fukushima

Department of Pediatrics, Keio University School of Medicine, Tokyo (K.M., T.O., N.M.); Department of Pediatrics, Tokyo Metropolitan Kiyose Children's Hospital, Tokyo (T.N.); Department of Pediatrics, Hiroshima University School of Medicine, Hiroshima (N.S.); Department of Hygiene and Medical Genetics, Shinshu University School of Medicine, Matsumoto, Japan (Y.F.); Telethon Institute of Genetics and Medicine, Milano, Italy (B.F., A.B.); and Institut für Humangenetik, Ruprecht-Karls-Universität Heidelberg, Heidelberg, Germany (G.R.)

Although genotype-phenotype correlations in male patients with various types of nullisomy for Xp22.3 have assigned a locus for X-linked mental retardation (MRX) to an approximately 3-Mb region between DXS31 and STS, the precise location has not been determined. In this paper, we describe a 14 7/12 year old Japanese boy with mental retardation and an interstitial deletion at Xp22.3 involving STS, KAL1, and OA1, and compare the deletion map with that of previously reported three familial male patients with low-normal intelligence and a similar interstitial deletion at Xp22.3. The results suggest that the MRX gene is further localized to the roughly 1.5-Mb region between DXS1060 and DXS1139.

KEY WORDS: mental retardation, X chromosome, X-inactivation, interstitial deletion, gene locus

INTRODUCTION

Genotype-phenotype correlations in male patients with various types of nullisomy for the Xp22.3 region have identified six contiguous disease genes for short stature (SS), X-linked recessive chondrodysplasia punctata (CDPX1), X-linked mental retardation (MRX), X-linked ichthyosis (XLI) due to steroid sulfatase (STS) deficiency, Kallmann syndrome (KAL1), and ocular albinism type 1 (OA1) [Ballabio and Andria, 1992; Schaefer et al., 1993]. The growth gene(s) for SS has been assigned to the distal part of the pseudoautosomal region and, therefore, SS is also manifested by female patients monosomic for the critical region for the pseudoautosomal growth gene(s) [P-growth gene(s)] [Ogata et al., 1992]. By contrast, the remaining five genes have been mapped to the X-differential region and, hence, these diseases are recognized as X-linked recessive traits [Ballabio and Andria, 1992; Schaefer et al., 1993].

For the five X-linked recessive disease genes, extensive molecular approaches have been carried out, successfully cloning the genes for CDPX1 [Franco et al., 1995], XLI [Ballabio et al., 1987; Bonifas et al., 1987; Conary et al., 1987; Yen et al., 1987], KAL1 [Franco et al., 1991; Legouis et al., 1991], and OA1 [Bassi et al., 1995]. Furthermore, several genes of unknown biological function, such as ARSD [Franco et al., 1995], ARSF [Franco et al., 1995], PKX1 [Klink et al., 1995], GS1 [Yen et al., 1992], GS2 [Lee et al., 1994], APXL [Schiaffino et al., 1995], and CLCN4 [van Slegtenhorst et al., 1994], have also been isolated from the X-differential region. However, the gene for MRX has not been cloned to date, although it has been assigned to an approximately 3-Mb region between *DXS31* and *STS* [Ballabio et al., 1989]. The GS1 gene has been shown to lie between *DXS31* and *STS*, but normal intelligence in male patients missing *GS1* argues against *GS1* being the MRX gene [Yen et al., 1992].

In this report, we describe a Japanese boy with mental retardation (MR) and an interstitial deletion at Xp22.3 involving *STS*, *KAL1*, and *OA1*, and discuss the location of the MRX gene between *DXS31* and *STS*.

CLINICAL REPORT

This boy was born to non-consanguineous parents at 40 weeks of gestation after an uncomplicated pregnacy and delivery. At birth, the length was 48.8 cm (mean –0.6 SD) and the weight was 2.8 kg (mean –1.1 SD).

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Address reprint requests to Dr. Tsutomu Ogata, Department of Pediatrics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan.

Shortly after birth, he exhibited ichthyosis, nystagmus, and photophobia. He also had developmental retardation: he developed head control at 6 months, and walked without support and spoke single words at age 2 years. Because of the developmental delay, a brain computed tomographic scan and an electroencephalogram were obtained at age 4 years, showing no abnormal findings. He was enrolled in a special school for mentally delayed children. His height remained around the –2 SD of the mean of normal Japnese boys in the childhood and became below the –2 SD of the mean from 11 years old because of the lack of pubertal growth spurt.

At age 14 7/12 years, the patient was seen at Tokyo Metropolitan Kiyose Children's Hospital. Physical examination showed short stature (137.5 cm, mean –4.8 SD), generalized ichthyosis, horizontal nystagmus, micropenis (length 3.0 cm, mean –6.6 SD), hypoplastic scrotum, bilateral cryptorchidism, and lack of pubic hair. There were no minor anomalies except for clinodactyly of the fifth fingers. Ophthalmologic examinantion revealed retinal hypopigmentation and foveal hypoplasia. His intelligence quotient (IQ) was assessed as 46 by the Tanaka-Binet method. Routine laboratory tests were normal, and endocrine studies showed hypogonadotropic hypogonadism: serum luteinizing hormone was 0.18 IU/L (age-matched reference value, 0.50–6.50 IU/L), follicle-stimulating hormone 1.25 IU/L (1.23–10.5 IU/L), and testosterone below 0.5 nmol/L (15.3 ± 6.0 nmol/L). STS activity determined by the high performance liquid chromatographic method [Ohsaki et al., 1993] was undetected in peripheral lymphocytes. Radiological studies for short stature disclosed no abnormal findings except for delayed bone age of 10 6/12 years.

The pedigree is shown in Figure 1. The father (II-1) was 165 cm tall (mean –0.8 SD), the mother (II-2) 158 cm tall (mean +0.2 SD), the 11-year-old sister (III-2) 135 cm tall (mean –1.5 SD), and the three-year-old sister (III-3) 89 cm tall (mean –1.3 SD). All the relatives were mentally normal: the parents were both medical doctors and the sisters had a good record at a standard school. STS activity of lymphocytes was 408.3 pmol/h/mg protein for the mother (II-2), 388.2 pmol/h/mg protein for the elder sister (III-2), and 553.7 pmol/h/mg protein for the younger sister (III-3) (normal female value, mean 964 pmol/h/mg protein, range 658–1,100 pmol/h/mg protein). The grandmother (I-2), the mother (II-2), and the elder sister (III-2) had mild photophobia.

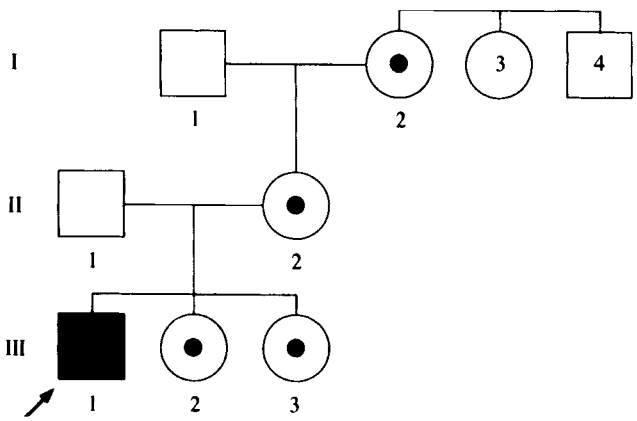

Fig. 1. Pedigree of the family.

METHODS

Cytogenetic Studies

G-banding chromosome analysis was performed on 30 peripheral lymphocytes of the patient and the mother. For the patient, high resolution G-banding was also carried out with ethidium bromide [Ikeuchi, 1984].

Molecular Studies

Polymerase chain reaction (PCR) and Southern blotting were employed for genomic DNA extracted from lymphoblastoid cell lines. For PCR analysis, genomic DNA of the patient and normal subjects was amplified with the primers defining following loci at Xp22.3 (from telomere to centromere): *PABX*, *CDPX1*, *DXS31*, *DXS89*, *DXS1060*, *DXS996*, *DXS1139*, *GS1*, *DXS1130*, *STS*, *KAL1*, *DXS143*, *DXS1137*, *OA1*, *DXS1140*, *DXS410*, *CLCN4*, and *DXS1136*. The primer sequences and the PCR conditions were as decribed previously [Schaefer et al., 1993; van Slegtenhorst et al., 1994; Bassi et al., 1995; Franco et al., 1995; Herrell et al., 1995]. For Southern blot analysis, the genomic DNA of the patient, the grandmother, the mother, the two sisters, and normal subjects was digested with *Eco*RI and hybridized with following probes defining different loci at Xp22.3 (from telomere to centromere): M1A (*DXS31*) [Mondello et al., 1987], ST-12 (*STS*) [Ross et al., 1990], dic56 (*DXS143*) [Middlesworth et al., 1985], 18-55 (*DXS70*) [Mondello et al., 1987], and 782 (*DXS85*) [Hofker et al., 1985]. The copy number of examined loci in the four females was determined by the comparison of band intensity between each locus and autosomal TK gene [Lau and Kan, 1984] as an internal control.

RESULTS

Cytogenetic Studies

The karyotype was 46,XY for the patient and 46,XX for the mother. High-resolution G-banding detected no structural abnormality of the X chromosome of the patient.

Molecular Studies

Representative results are shown in Figures 2 and 3, and the deletion map of the patient is depicted in Figure 4 (case 1). The patient was negative for the 11 loci from *DXS996* to *DXS410* including *STS*, *KAL1*, and *OA1*, and positive for the remaining nine loci analyzed. Dosage blottings indicated that, in all the four females examined, *STS* and *DXS143* were present in a single copy, whereas *DXS31*, *DXS70*, and *DXS85* were present in double copies.

DISCUSSION

The present study showed that our patient had a submicroscopic interstitial deletion with the breakpoints between *DXS1060* and *DXS996* and between *DXS410*

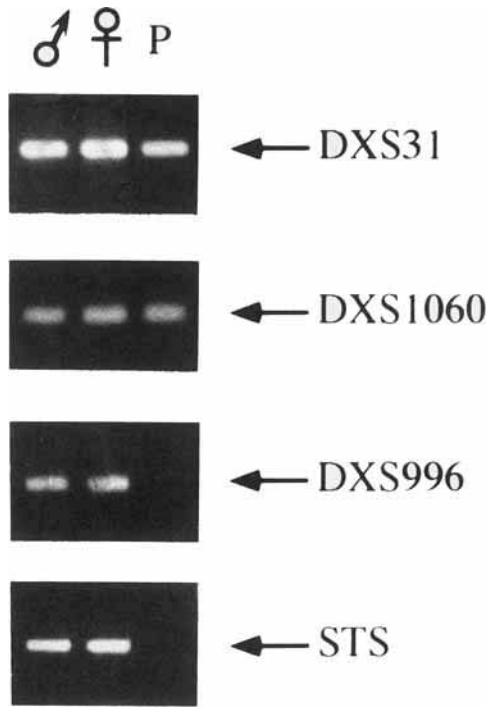

Fig. 2. Representative results of polymerase chain reaction analysis. The patient is positive for *DXS31* and *DXS1060*, and negative for *DXS996* and *STS*.

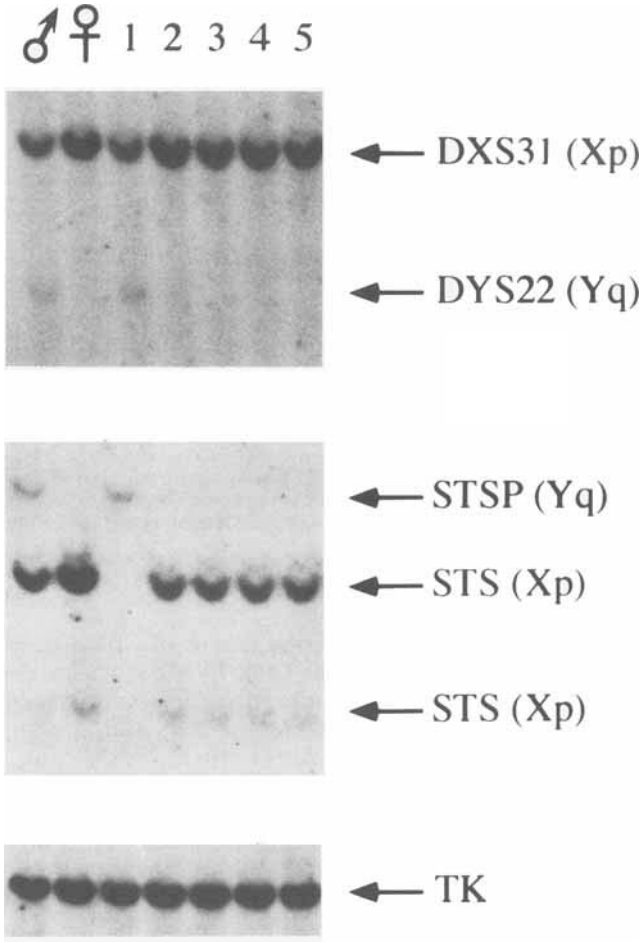

Fig. 3. Southern blot analysis (♂, normal male; ♀, normal female; 1, patient; 2, grandmother; 3, mother; 4, elder sister; 5, younger sister). Shown are *Eco*RI digests hybridized with M1A defining *DXS31* at Xp22.3 and *DYS22* at Yq (**top panel**), ST-12 defining *STS* at Xp22.3 and *STSP* at Yq (**middle panel**), and the probe for autosomal TK gene (**bottom panel**) (same filter). In the patient, *DXS31* is preserved and *STS* is deleted. Comparison of the band intensity between *DXS31*, *STS*, and *TK* indicates that, in the four females examined (2–5), *DXS31* is present in two copies and *STS* is present in a single copy.

and *CLCN4*, and that the deletion was shared by the grandmother, the mother, and the two sisters. Since the deletion involves *STS*, *KAL1*, and *OA1*, it explains the skin lesion, the hypogonadotropic hypogonadism and resultant events, e.g., infantile external genitalia with cryptorchidism, lack of pubertal growth spurt, and delayed bone age, and the eye lesion of our patient. Although our patient had SS in the probable presence of the P-growth gene(s), the SS may be ascribed to the combined effects of several factors such as chromosome imbalance, absence of multiple genes, and severely compromised androgen production [Aynsley-Green et al., 1976; Gilbert and Opitz, 1982]. Consistent with the presence of the P-growth gene(s), the carrier females heterozygous for the deletion were normal in height.

An important manifestation in our patient is moderate to severe MR. This indicates that an MRX gene is deleted or disrupted in our patient. One may argue that an adequate mental assessment in terms of the MRX gene is difficult in the presence of complex phenotype, and that factors other than loss of the MRX gene, such as chromosome imbalance and loss of multiple genes, could lead to MR [Gilbert and Opitz, 1982]. In this context, three male patients in a pedigree described by Sunohara et al. [1986] are informative. The three patients had complex phenotype compatible with loss of *STS*, *KAL1*, and *OA1*, but were free from obvious MR (IQ was 81, 90, and 91, respectively). Schaefer et al. [1993] reported that the patients had an interstitial deletion with the breakpoints between *DXS996* and *DXS1139* and between *DXS1140* and *DXS410* (Fig.4, case 2). The comparison of our patient with the three previously reported patients suggests that our patient has more severe MR than the other three, despite the effects of other factors such as chromosome imbalance and loss of multiple genes being similar. Thus, although the deletion of the region around *DXS410* in our patient may also contribute to the difference in the mental status between our patient and the three patients, it appears reasonable to assume that the MRX gene is present in the region between *DXS1060* and *DXS1139* which is about 1.5-Mb long [Ferrero et al., 1995], and that the gene is impaired in our patient and preserved in the three patients. Furthermore, the moderate to severe MR of our patient may be explained by the co-existence of the loss of the MRX gene and the effects of other factors such as chromosome imbalance and deletion of other genes, and the low-normal intelligence of the three patients may be accounted for by the

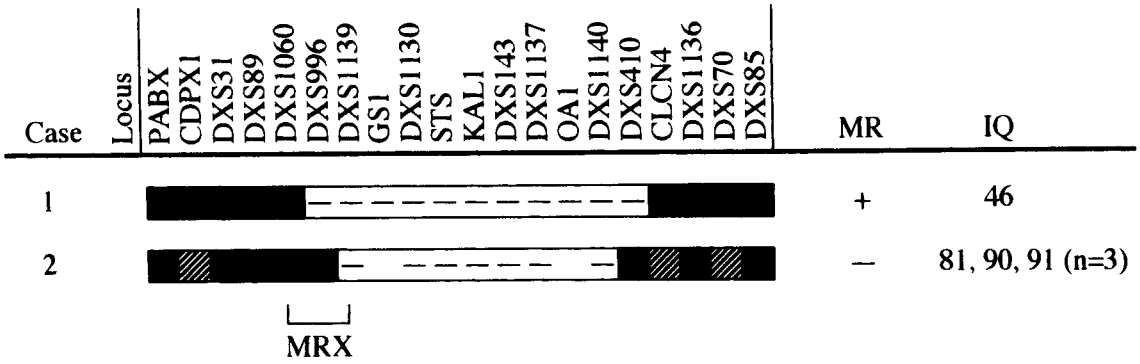

Fig. 4. Deletion map and mental status in our patient (case 1) and three male patients reported by Sunohara et al. [1986] and Schaefer et al. [1993] (case 2). MR, Mental retardation; IQ, Intelligence quotient. Black segments represent the positive loci confirmed by molecular studies, striped segments the presumably positive loci inferred from interpolation, minus symbols the negative loci cofirmed by molecular studies, and open segments the presumably negative loci inferred from interpolation. The locus order is based on the reports of Schaefer et al. [1993], van Slegtenhorst et al. [1994], Bassi et al. [1995], Franco et al. [1995], and Herrell et al. [1995]. The location of the MRX gene is indicated.

effects of factors other than the loss of the MRX gene. To our knowledge, there has been no other report describing a patient with an interstitial deletion that has the breakpoint between *DXS31* and *STS* and involves *STS*, *KAL1*, and *OA1*.

The putative MRX gene is expected to escape X-inactivation, as has been demonstrated for the genes for CDPX1 [Franco et al., 1995], STS [Ropers et al., 1981], and KAL1 [Franco et al., 1991]. Since *OA1* is believed to undergo X-inactivation [Lang et al., 1990], the mild photophobia in the heterozygous carrier females indicates that the normal X and the deleted X undergo random, rather than non-random, X-inactivation. Thus, if the MRX gene is subject to X-inactivation, it appears inexplicable why the carrier females are quite normal in mental development. In this case, the MRX gene is present in two active copies in normal females and in a single active copy in the carrier females, but the dosage difference may have no clinical effect, as has been observed for the genes *CDPX1*, *STS*, and *KAL1* [Ballabio and Andria, 1992]. Indeed, normal males have a single active copy of the MRX gene.

In summary, we propose that the MRX gene may be present in the roughly 1.5-Mb region between *DXS1060* and *DXS1139*. However, since MR is a highly heterogeneous phenotype, it might be possible that MR of our patient is caused by some factor(s) other than the interstitial deletion at Xp22.3. It is also possible that our patient, or the three previously reported patients [Sunohara et al, 1986; Schaefer et al., 1993], have an undetected complex deletion. Further accumulation of informative patients will permit a more precise localization of the MRX gene.

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